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Dose-response relationships between pollination and fruiting refine pollinator comparisons for cranberry (*Vaccinium macrocarpon* [Ericaceae])[1]

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Comparisons of pollinator efficacy using pollen received on stigmas can be refined by incorporating experimental dose–response relationships for pollen deposition and fruiting responses. A range of discrete pollen doses applied to cranberry stigmas resulted in decelerating curvilinear responses for fruiting, berry size, and seed set. Minimum thresholds and maximum asymptotes bounded reproductive responses to incremental stigmatic pollen loads. Four bee species were compared for their pollination efficacies on commercial cranberries, using counts of pollen received by stigmas during single bee visits to previously virgin flowers. Differences between these bee species were found to be exaggerated when raw pollen counts were used for comparison because foragers of some species often delivered pollen in excess of that needed to maximize fruit and seed production. Sixfold differences between species in mean pollen deposition translated into 1.5–2-fold differences in predicted cranberry fruit set and size. Implications for pollen tube competition and agricultural production are discussed.

Key words: Apiformes; bees; Ericaceae; fruit set; pollination efficacy; pollinator; stigmatic load; *Vaccinium macrocarpon*.

Sexual reproduction by many agricultural crops and wild flowering plants depends upon floral visitors, especially insects such as bees, to move pollen between compatible, receptive flowers. Species of floral visitors often differ in their contributions to pollination, both in their frequencies of floral visitation and their efficacies as pollinators. Pollination efficacies of floral visitors are commonly compared by controlling pollinator visits to flowers and measuring either resultant receipt of pollen by stigmas or else fruit set (e.g., Motten et al., 1981; Tepedino, 1981; Spira et al., 1992; Murali, 1993; Bosch and Blas, 1994; Javorek et al., 2002). Limitations accompany both methods.

The seemingly more direct measure of reproductive response to pollination tracks fruits or seeds developing from singly (or sequentially) visited flowers (e.g., Motten et al., 1981; Parker, 1981; Tepedino, 1981; Cane et al., 1985; Bosch and Blas, 1994; Sampson and Cane, 2000). A drawback of this method is that post-pollination processes can subtract from potential fruit and seed set. For example, despite adequate pollination, a developing fruit may abort if limited maternal resources are usurped by neighboring fruits (e.g., Stephenson, 1981; Helenurm and Schaal, 1996; Corbet, 1998), leading to an underestimation of pollinator efficacy. In addition, it is often impractical to await fruit maturation in the field before evaluating pollination treatment. Additional visits by pollinators must be prevented until flowers senesce, and pedicels must be labelled with durable tags that can be found months later (Cane, 1991). Fruits and plants are also at considerable risk for herbivory, frugivory, seed predation, disease, and physical damage, especially in wildland settings. All of these post-pollination processes will diminish pollinator efficacy estimates and obscure differences between pollination treatments.

A more immediate and dependable method for assessing pollinator efficacy uses counts of the number of compatible, viable pollen grains acquired by a virgin stigma during a single pollinator visit to a flower. Pollinator efficacies are then traditionally calculated from the mean or median stigmatic pollen loads counted on stigmas (e.g., Tepedino, 1981; Spira et al., 1992; Cane et al., 1996; Javorek et al., 2002). Differences in pollen delivered to stigmas can be attributed to pollinators' taxonomic identities or such factors as sex, body size, or handling behavior (e.g., Tepedino, 1981; Cane et al., 1996; Bingham and Ort, 1998; Freitas and Paxton, 1998; Gross and Mackay, 1998; Thomson and Goodell, 2001; Javorek et al., 2002). Comparisons using stigmatic pollen loads have the added advantage of isolating the contributions made by these pollinator attributes from other, post-pollination processes, such as pollen tube competition or maternal resource competition.

Stigmatic pollen load can serve as a convenient and seemingly uncomplicated proxy measure for fruit and seed set if relationships can be developed between stigmatic pollen doses and fruiting responses. However, stigmatic pollen loads may not translate directly and linearly into the probability of fruit set, the size of fruits, or counts of viable seeds. There is also the common dilemma of distinguishing self- from outcross pollen for self-incompatible species (Snow, 1982). Self-pollen is often barred from fertilizing ovules by self-incompatability mechanisms, but for only a few agricultural cases have observable characters been found to distinguish self- from outcross pollen (Degrandi-Hoffman et al., 1992). Differences in the counts of pollen received from different pollinators at self-incompatible plant species must therefore be interpreted cautiously.

Even if the plant of interest is self-compatible, the relation-

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ship between the number of pollen grains deposited and fruit set is likely to be a nonlinear saturation function (Harder and Thomson, 1989). Enough pollen must be received to exceed a minimum threshold for fertilizing sufficient ovules to elicit fruit set (Stephenson, 1981; Birrenkott and Stang, 1989). Additional pollen often results in larger, faster ripening, more seedy fruits that are less likely to abscise prematurely (Schlichting et al., 1987; Winsor et al., 1987; Falque et al., 1995) and whose seed may be more vigorous (Hawthorne et al., 1956; Richardson and Stephenson, 1992; Quesada et al., 1993; Cruzan and Barrett, 1996). Pollen in excess of that needed to fertilize all ovules is superfluous if not sometimes detrimental (reviewed in Young and Young, 1992), except for cases of inter-male competition (Snow and Spira, 1991; Quesada et al., 1993; Cruzan and Barrett, 1996). Thus, in terms of fruit and seed set, a visitor depositing twice as many pollen grains as are needed to fertilize all ovules should be equivalent to another pollinator species that consistently deposits the minimum needed to fertilize all ovules. Conversely, classes of floral visitors that regularly deliver some pollen grains during a single visit but never enough to elicit fruit set may not be pollinators at all without repeated visitation (Stanghellini et al., 1998). Estimating pollination efficacy for different floral visitors could be improved if pollen loads on stigmas could be translated into fruit and seed sets for a given plant species. A series of such studies might reveal broader predictive relationships between stigmatic load and fruit and seed production.

In this pollination study of cranberries (*Vaccinium macrocarpon* Aiton [Ericaceae]), we experimentally explored the relationship between stigmatic pollen load and fruiting. We then applied these empirical relationships to comparisons of pollinator efficacy. Receptive flowers of greenhouse plants were manually pollinated with predetermined doses of pollen tetrads. Resulting mature fruits were later harvested. The numbers of tetrads placed on stigmas were related to characteristics of the mature fruits and seeds. We then compared the pollination efficacies of different bee species in the field by counting the number of pollen grains received by virgin cranberry stigmas during single visits by freely foraging honey bees (*Apis mellifera* L.), bumble bees (*Bombus affinis* Cresson), and females of two species of leafcutting bee (*Megachile addenda* Cresson and *M. rotundata* Fab.). We contrasted the traditional method for comparing pollinator efficacies—mean numbers of pollen grains delivered—with the estimated yields of fruits and seeds calculated to result from their floral visits using the regressions developed in the manual pollination experiment.

MATERIALS AND METHODS

Natural history of cranberry pollination—The American cranberry (*Vaccinium macrocarpon*) is a perennial trailing woody vine that is native to bogs, swamps, shorelines, and streambanks of northeastern North America. Cranberry blooms during June and July, bearing ranks of solitary flowers along short upright shoots that grow above the mat of vines (Eck, 1986). Ramets of *V. macrocarpon* are largely self-fertile, although outcrossing sometimes yields marginal gains in seed set (Sarracino and Vorsa, 1991; MacKenzie, 1994). Vigorous vegetative multiplication has facilitated its cultivation during the 20th century.

Cranberry flowers are white, hermaphroditic, and possess basal nectaries and a staminal column. The nectar of fresh flowers contains 300–400 μg of dissolved sugar (Cane and Schiffhauer, 1997); individual flowers produce ca. 7000 pollen tetrads (Cane et al., 1996). Each tetrad consists of four pollen grains. Ovaries of the 'Stevens' cultivar studied here contain 32.5 ± 4.2 ovules (Sarracino and Vorsa, 1991). The stigma of the single pistil becomes viscid when receptive and is exserted beyond the anther tips, thus limiting opportunities for autogamy. Anthers dehisce their pollen tetrads through paired terminal pores. In contrast with the urceolate corolla derived from fused petals that typify many *Vaccinium* species, the cranberry corolla consists of four strap-like petals that are reflexed at floral maturity, revealing the beak-like staminal column. Unvisited flowers rarely produce fruit, even when mechanically shaken (as by wind) (MacKenzie, 1994).

Bees always probe cranberry flowers for nectar. Simultaneously, some foragers will vibrate the staminal column for pollen as they hang from the pendant flower. To release pollen, they either shiver their thoracic flight muscles to sonicate the flower (e.g., *Bombus*) or drum the staminal column using their mid- or hind legs (e.g., *Megachile* and *Osmia*) (Cane et al., 1996). For unknown reasons, honey bees instead use their forelegs to drum the staminal column for pollen (Cane et al., 1993), which precludes simultaneous probing for nectar. Honey bees may probe cranberry flowers for nectar either legitimately, parting the staminal tips with their inserted proboscis, or on occasion illegitimately, by inserting their proboscis between the bases of two staminal filaments. Illegitimate foragers make no stigmatic contact.

Stigmatic loads needed to set fruit and seed—*Manual pollination*—Upright shoots of the cranberry cultivar 'Stevens' were cut from a producing bog during July 1995 and rooted individually in forestry propagation cells (''Conetainers'' from Stuewe and Sons, Corvallis, Oregon, USA). 'Stevens' is a cross of two wild accessions (Sarracino and Vorsa, 1991). Rooting and growing media were a standard mix of sand and peat used in cranberry propagation. Plants were allowed to go dormant in an unheated greenhouse during the fall of 1995 and then moved to a heated greenhouse during March 1996. These plants were experimentally pollinated in 1996 and 1997.

One flower per rooted cutting was emasculated before opening to prevent any autopollination. All other flowers were removed. Flowers were deemed receptive when fluid was visible on the stigma, typically 4–5 d after emasculation. On each pollination date, donor pollen was gathered on a glass slide from open flowers of the cultivar 'Early Black', a commonly grown, effective pollenizer for 'Stevens' (Sarracino and Vorsa, 1991). Outcrossing was chosen to avoid any subtle deleterious consequences of geitonogamy (e.g., MacKenzie, 1994). Individual recipient plants were inverted under a stereomicroscope so that the emasculated flower was visible. Pollen tetrads were transferred individually to a stigma using a single stiff hair. Experimental stigmatic pollen loads consisted of 0, 2, 4, 8, 16, or 32 pollen tetrads. Pollen loads were randomly assigned to individual flowers within each year's experiment. After pollination, plants were held in the greenhouse until fruits matured. Ninety days after pollination, ripe fruit were removed, weighed, and dissected for mature seeds.

We used nonlinear regression to evaluate relationships between applied stigma load and mean fruit set, seed set, and berry size. We used logistic regression to assess the probability of fruit set with incrementally increasing stigmatic loads (SAS, 1989). The normit link function yielded the best model fit statistics and model convergence. The mean values for fresh fruit mass and seed counts were regressed on the counts of pollen tetrads applied to stigmas, using nonlinear regression to develop best fit equations (SigmaPlot 4.0; SPSS, Chicago, Illinois, USA). Seed and pollen counts were square-root transformed to achieve acceptable normality (Wilk-Shapiro test; $P > 0.05$). Since maturing seeds are known to hormonally direct fruit development, seed count was loaded into the stepwise regression model first, to test the hypothesis that variation in fruit mass responds to stigmatic load independent of seed count. These equations were used to predict expected fruit sets, fruit masses, and seed counts from the number of pollen tetrads deposited during a single floral visit by a bee.

Pollination by freely foraging bees—We compared the numbers of pollen tetrads deposited by four bee species during single visits to virgin cranberry flowers. Comparisons were made at two bogs near Chatsworth, New Jersey, USA, with blooming 'Early Black' cranberries. A week prior to the experiment, we used polyester floating row covers to exclude floral visitors from four 8-m^2 patches of blooming cranberries on the bogs. Row covers were also

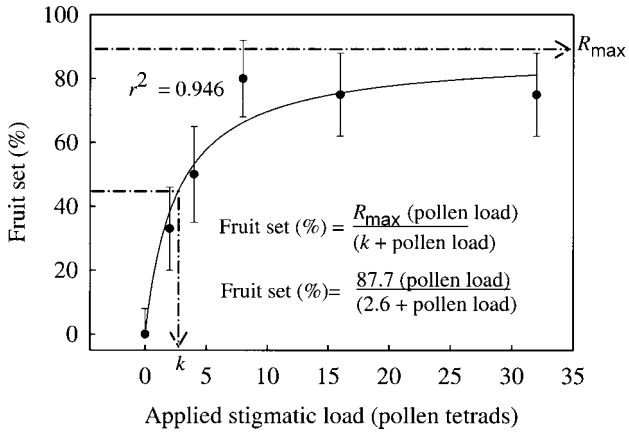

Fig. 1. Hyperbolic regression for the probabilities of fruit set resulting from a series of incremental pollen loads placed on receptive stigmas of flowering cranberry plants (means ± 1 SD). One flower pollinated per upright shoot on rooted greenhouse cuttings of the cultivar 'Stevens'. Sample sizes range from 43 to 48 flowers treated per dosage class.

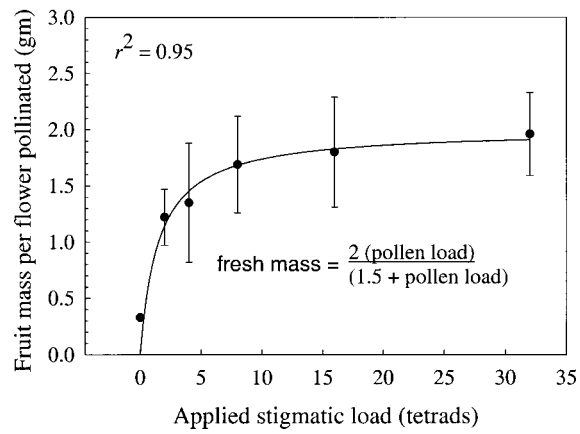

Fig. 2. Hyperbolic regression for the fresh masses of ripe cranberries resulting from the controlled pollinations of flowers described in Fig. 1 (means ± 1 SD).

placed over half of the vines in each of two 48-m^3 flight cages that had been set up on one of the bogs just prior to bloom.

At the start of an experiment, a row cover was pulled back to allow foraging bees access to these virgin flowers. Freely foraging *Bombus affinis* were sufficiently abundant on the bogs for us to readily accumulate single visits to flowers at the open experimental patches. For honey bees, a five-frame nucleus colony was moved to one of the field cages. Worker bees were allowed to forage at unmanipulated flowers for 5 d prior to experiment. Confinement deprived the colony of alternative pollen sources, thereby favoring pollen-harvesting from cranberry. Mated female *M. addenda* from elsewhere on the bog were induced to nest in the second field cage. Standard nesting blocks for *M. rotundata*, a non-native managed bee species not naturally found on cranberry, were placed in this field cage too. Freshly emerged, mated females were allowed to forage on unmanipulated flowers for 5 d prior to experiment. By this time, all female *Megachile* were collecting cranberry pollen and nectar to provision their leaf-lined nests. Hence, all females of these four bee species visited live reserved flowers during undisrupted foraging bouts in the field.

Virgin flowers receiving single legitimate visits from bees were gently clipped, inverted, and placed in a well of a tissue culture plate and returned to the laboratory. At the same time, additional unvisited neighboring flowers were taken to serve as controls for autopollination. Flowers were classed as vibrated by pollen foragers, visited for nectar only, or unvisited. Flowers visited without stigmatic contact were discarded (most cases involved honey bees or *M. rotundata*). In the laboratory, stigmas were squashed individually on microscope slides in acetocarmine jelly and the total numbers of pollen tetrads counted.

We compared the counts of cranberry pollen tetrads received by stigmas following single visits from each bee species. We also calculated estimates for fruit set, fruit mass, and seed set from these raw data for stigmatic loads, using the functional relationships between pollen deposition and each of these variables that were derived from the manual pollination experiments. Variances of single-visit treatments were homogeneous (Levene's test, $P \geq 0.05$) (Sokal and Rohlf, 1995) once the calculated seed counts were transformed to their square roots. Because of the excess of zero values, data for unvisited flowers could not be satisfactorily transformed, so stigmatic loads of virgin flowers (the result of any autopollination) were compared separately to just those resulting from single visits by honey bees (which delivered the fewest pollen tetrads during single visits) using the nonparametric Wilcoxon two-sample test (U_s). The four bee species were then compared for delivered stigmatic loads and estimated fruit set, fruit mass, and seed set using an ANOVA followed by GT2 a posteriori comparisons (owing to large differences in sample sizes [Sokal and Rohlf, 1995]) (SAS, 1989).

RESULTS

Stigmatic loads needed to set fruit and seed—*Manual pollination*—The probability of fruit set (Fig. 1), the mass of resultant cranberry fruit (Fig. 2), and the number of mature seeds (Fig. 3) increased as a function of the incremental number of pollen tetrads manually placed on each stigma. Simple hyperbolic equations yielded excellent fits to the means of the empirical data. Percentage fruit set did not increase with >8 tetrads (Fig. 1). Berry mass increased only marginally with >8 tetrads (Fig. 2). Seed set improved with incremental stigmatic pollen loads of up to 16 pollen tetrads (Fig. 3). Thus, our range of 0–32 pollen tetrads per stigmatic load bracketed the entire range of possible fruit set, fruit size, and seed set responses measured using our greenhouse protocol.

Stigmatic pollen load explained a significant proportion of the variation in fruit set and yield of fruit mass. Over a range of 0–16 pollen tetrads, the relationship between percentage fruit set and the number of pollen tetrads placed on stigmas was significant by logistic regression (likelihood ratio $\chi^2 = 5.16$, $P = 0.02$); with the addition of data for 32 pollen tetrads, the relationship was no longer significant. Multiple regression showed that fresh mass of cranberries increased with both the

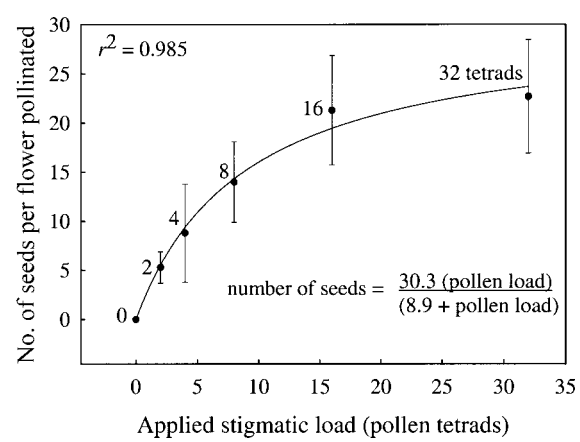

Fig. 3. Hyperbolic regression for the content of mature, dark seeds from ripe cranberries resulting from the controlled pollinations of flowers described in Fig. 1 (means ± 1 SD).

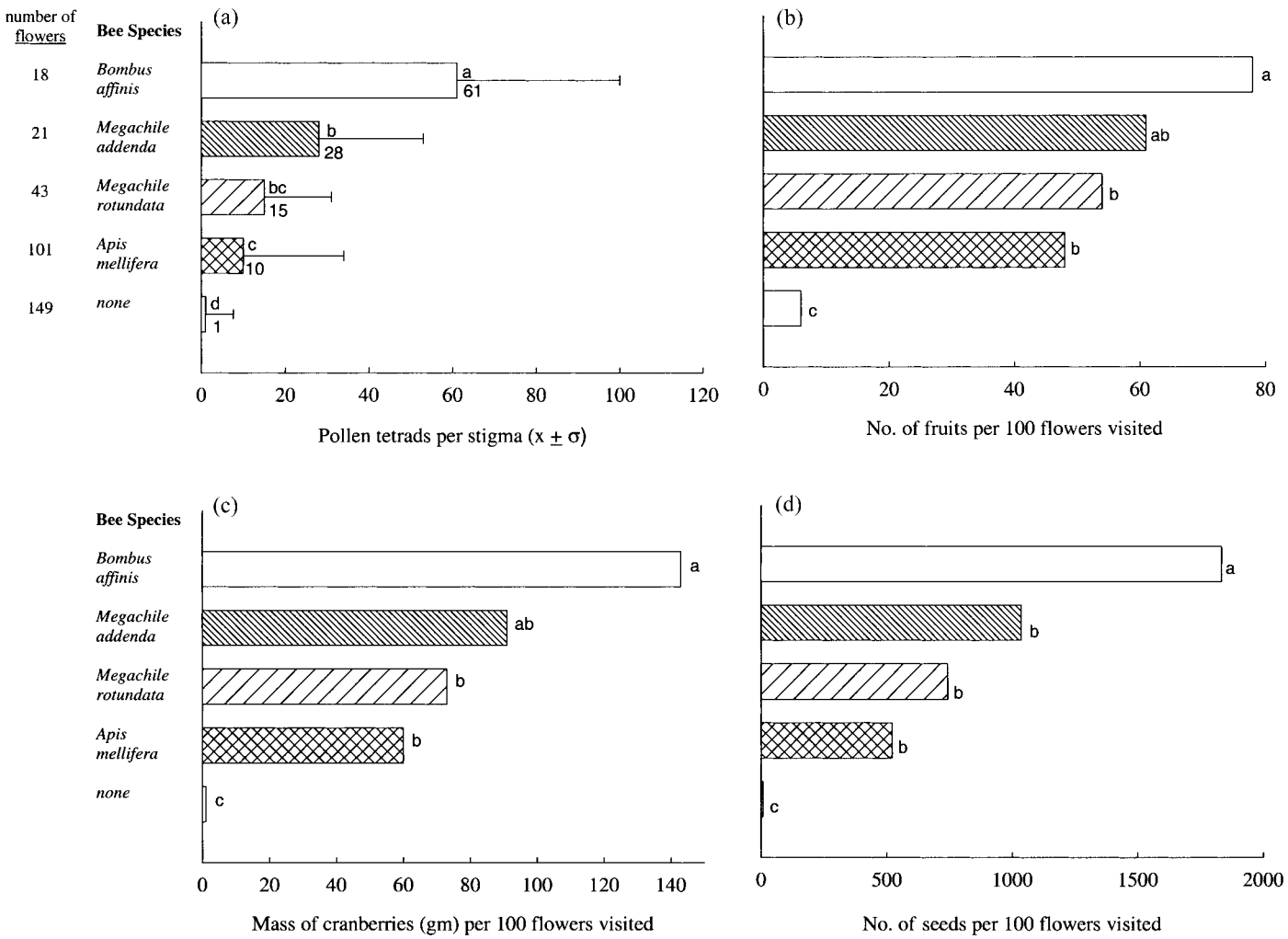

Fig. 4. Differences between bee species in single-visit pollen deposition and predicted fruit set, berry mass, and seed content of resulting cranberries. (a) Counts of cranberry pollen remaining on stigmas of virgin flowers visited once by freely foraging bees on a blooming cranberry bog (means ± 1 SD). For 100 visited flowers, each bee species is compared for the predicted production of cranberry fruits (b), the total mass of those fruits (c), and their complement of seeds (d); calculations were made using the regression equations presented in Figs. 1, 2, and 3. Bars with different letters are statistically different.

number of pollen tetrads applied to stigmas and the seed content of the fruit ($F_{2,132} = 43.7$, $P \leq 0.0001$). Once the covariation between seed count and mass of fruit was removed from the analysis, stigmatic load was no longer predictive of fruit mass ($F_{1,132} = 1.1$, $P = 0.29$).

Pollination by freely foraging bees—On average, individual foragers of all bee species deposited sufficient pollen to set fruit for at least half of the flowers that they visited (Fig. 4b). Flowers visited once by honey bees received the least pollen compared with visits from other bee species, but these stigmas nevertheless received 10-fold more pollen than stigmas of unvisited flowers ($U_s = 18\,120$, $t \leq 0.0001$) (Fig. 4a). Hence, individual legitimate visits by honey bees added significantly to the pollen load of a virgin flower.

The number of cranberry pollen tetrads received by virgin stigmas during single visits differed among bee species ($F_{3,179} = 22.8$, $P \leq 0.0001$). The estimated probabilities of fruit set, estimated fruit mass, and estimated number of mature seeds also differed with the species of pollinating bee ($F_{3,179} = 4.2$–7.3, $P \leq 0.007$–0.0001) (Fig. 4). Bumble bees were superior to both honey bees and *M. rotundata* in all measures of pollination efficacy; the other leafcutting bee, *M. addenda*, was intermediate in value. Bee species that, on average, delivered more pollen to cranberry stigmas during single visits also more frequently delivered pollen in sufficient quantities to maximize the likelihood for fruit set (Fig. 5). However, the differences between bee species in estimated fruiting responses were much less or insignificant compared with the number of pollen tetrads that they deposited on stigmas (Figs. 4 and 5).

DISCUSSION

Cranberry fruit set, fruit mass, and seed number increased with the amount of conspecific pollen received by a cranberry flower's stigma. Manual pollinations with discrete increments in pollen dosage revealed that these relationships were best described by a family of first-order rectangular hyperbolas characterized by (1) a minimum threshold and (2) a maximum asymptote beyond which additional pollen did not enhance fruiting or seed production (Figs. 1, 2, and 3). Bee species differed significantly in the mean amounts of pollen that they

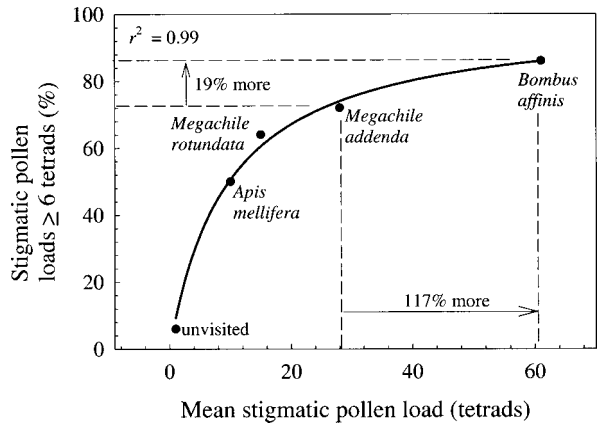

Fig. 5. Curvilinear regression comparing bee species for the mean stigmatic loads that they delivered to the proportions of those visits that left sufficient pollen to maximize fruit set on cranberry. Stigmatic loads were taken following single visits to virgin flowers by freely foraging bees (Fig. 4a). The sufficiency of a pollen load to maximize fruit set was derived from greenhouse data presented in Fig. 1. The dashed lines contrast the discrepancy, when comparing pairs of pollinator species, between using mean stigmatic load of pollen vs. the estimated amount of pollen needed to maximize fruit set.

deposited on stigmas of visited cranberry flowers (Fig. 4a). Comparisons of simple pollen counts on cranberry stigmas generally overestimated the magnitudes of differences in pollinator efficacy, however, mostly because some bee species (especially *B. affinis*) delivered pollen far in excess of that needed to maximize cranberry fruit and seed production. Differences among pollinator species in pollination efficacy were more realistically compared using predicted cranberry reproductive responses interpolated using the regression equations from our manual pollination experiments (Fig. 4b–d). Following such transformation, species of cranberry pollinators retained their same rank order for mean amounts of pollen deposited on stigmas, but the magnitude of differences between pollinator species was diminished and in some cases rendered insignificant (Figs. 4 and 5).

Among self-compatible plants like cranberry, efficacies of pollinators have been profitably compared using pollen received by stigmas during single pollinator visits to flowers. However, by merely comparing the mean counts of pollen that stigmas receive from different pollinator species during their floral visits, identical simple linear relationships of pollen load with fruiting and seed set responses have been tacitly assumed, each with the same monotonically increasing slope of one, *y*-intercept of zero, and lack of an asymptote. These assumptions are of course unrealistic though rarely recognized when comparing pollination efficacies of different floral visitors. Raw counts of deposited pollen are more realistically compared following transformation into predicted plant reproductive responses (e.g., fruit and seed set, and fruit size). We were able to experimentally derive these relationships for cranberry using a family of first-order hyperbolic curves that fit cranberry reproductive responses to incremental doses of pollen received by stigmas.

Stigmatic loads needed to set fruit and seed—*Manual pollination*—Hyperbolic curves proved desirable for representing a cranberry plant's reproductive responses to variable pollination (Figs. 1–3). Hyperbolic curves offer a simple yet effective way to faithfully represent the three salient reproductive responses to pollination: fruit set, fruit size, and seed set. First-order hyperbolic curves require only arithmetic operators for their calculation. The general equation for this dose–response relationship can be represented by

$$r = \frac{R_{\max}(P_i)}{k + P_i}$$

where $R_{\max}$ = the maximum or asymptotic value for a given reproductive response (e.g., most seed, largest fruit, etc.), P_i = the actual stigmatic pollen load, k = the pollen load that gives ½ of $R_{\max}$, the maximum reproductive response, and r = calculated reproductive response for the given pollen load. Knowing these three variables, the resulting curve gives (1) a minimum threshold for stigmatic pollen load (the point for which ovule fertilization exceeds the minimum to avert fruit abortion), (2) a decelerating slope, and (3) the asymptote beyond which additional pollen confers no further gains in fruit set, fruit size, or ovule fertilization. For stigmatic loads, the gain in reproductive response typically decelerates with increasing pollen load. As pollen loads increase, male gametes are less and less likely to encounter an unfertilized ovule, either because fertilization is simply progressing toward saturation or perhaps because when pollen tubes crowd the style, they interfere with each other's elongation (Young and Young, 1992). Semi–in vivo and in vivo experiments with cranberry demonstrate the existence of both incomplete pollen germination and pollen tube attrition (J. H. Cane, unpublished data), which would explain why each pollen tetrad yielded far less than four seeds.

The shape of the rectangular hyperbola is given by just two variables, $R_{\max}$ and k, which suggests opportunities for deriving the relationships between reproductive responses and stigmatic pollen loads without the laborious experiments reported here. For example, $R_{\max}$ for seeds could be approximated as the number of available ovules per flower, the maximum seed content in fruits from thoroughly pollinated flowers, or the mean of the two measures. For cranberry seed set, the $R_{\max}$ calculated from our data is 30.3 seeds (Fig. 3), which agrees well with both the 32 ± 4 ovules reported for 'Stevens' (Sarracino and Vorsa, 1991) and the content of the seediest cranberry from our experiments (31 seeds). Maximum seed set could be sought among the largest fruits, which could also supply a value for maximum fruit size. Knowing just $R_{\max}$ is sufficient if one's objective is to merely truncate the distribution of stigmatic pollen loads at the asymptote, beyond which additional pollen is superfluous for further reproductive gain by these various measures (as in the comparison of *B. affinis* with *M. addenda*).

The pollen load that yields half of the asymptotic reproductive response, k, will likely have to be estimated empirically. For seed set of 'Stevens' cranberries, it would be the pollen load that yields 15 seeds ($R_{\max}/2$). One approach for determining k could involve pollination of tagged flowers, and once ovules have been fertilized (usually 24–48 h following pollination), removal and preservation of stigmas and their pollen loads (e.g., Falque et al., 1995). Mature fruits of intermediate size that result from these pollinations then could be weighed and dissected for seed. The ratio found between pollen load and fruit set, fruit size, and/or seed count for these tagged mid-sized fruits could then be used to estimate k, as the slope of the tangent to the curve. For instance, through manual polli-

nations of *Theobroma cacao*, Falque et al. (1995) experimentally demonstrated that the asymptotic maximum for fruit set is 85% (R_{max}), achievable with 115 pollen grains, and that 43 pollen grains yields half of this value (k). Once R_{max} and k are thus determined, the appropriate first-order hyperbolic curve can then be produced and used to interpolate the values for reproductive responses that will result from all other counts of pollen on stigmas.

Pollination by individual bees—Cranberry flowers in this study were clearly not autogamous under field conditions (Fig. 4); bees are needed for pollination, as has been shown before (reviewed in MacKenzie [1994]). The bee species that commonly visited cranberry flowers in this study differed in their efficacies as pollinators (Fig. 4). Interpretation of these differences in pollinator service, however, partly depends upon the plant reproductive response(s) of interest. This is a consequence of differences in the hyperbolic relationships between the loads of pollen received by stigmas and the three reproductive responses of interest.

Foraging workers of the bumble bee *B. affinis* were statistically superior to the other three pollinator species in both pollen delivered and the yield of seed. The larger pollen loads that were more frequently delivered by this bumble bee were therefore meaningful for boosting seed set. In contrast, such large pollen loads were superfluous for enhancing fruit production, as they exceeded asymptotic values. As a consequence, the lesser mean stigmatic pollen loads received from *M. addenda* were equivalent to those from *B. affinis* for predicted fruit set and mass (Fig. 4b–c). Honey bees were inferior to both of these pollinators in terms of pollen delivery to stigmas during single visits, but this does not mean that they are irrelevant for cranberry pollination, as has been previously asserted (e.g., Kevan et al., 1983). These modest pollen loads delivered to stigmas during legitimate floral visits by honey bees would yield nearly a 50% fruit set and considerable fruit and seed production in our system (Fig. 4b–c), simply because only 1.5–8.9 tetrads are needed to achieve 0.5 R_{max} ($=k$) for these three measures of cranberry reproductive responses to pollination (Figs. 1, 2, and 3). Considerable differences among these pollinators in the traditional measure of pollinator efficacy—mean stigmatic pollen load—diminish when stigmatic loads are translated into predicted reproductive responses of the plant in our simplified system (Fig. 5).

For honey bees specifically, a key factor for their performance as cranberry pollinators will be the proportions of nectar robbers and pollen foragers. Nectar robbers did not contact cranberry stigmas, but pollen foragers always did. Honey bee foragers that made stigmatic contact proved to be satisfactory cranberry pollinators in our study. We encouraged their foraging for pollen in our experiment by leaving the small nucleus colonies with paltry pollen provisions but hungry brood; confinement in a cage set over the cranberry bog forced them to forage on the cranberry flowers. The numbers of cranberry pollen foragers fielded by a hive is a function of two manageable variables, a colony's brood population and queen genotype (Cane and Schiffhauer, 2001). Populous colonies with many larvae and nurse bees demanding protein-rich pollen will field an abundance of pollen foragers if pollen stores are limited. So will colonies headed by queens selected for pollen-hoarding, a heritable quantitative trait (Cane and Schiffhauer, 2001). Insight gained from our study should shift the emphasis from pollination efficacy of honey bees to management that enhances the proportion of foragers that visit cranberry flowers and make stigmatic contact.

Implications—Commercial cranberry growers seek to maximize tonnage of fruit per hectare of bog in a cost-effective manner. A yield component analysis of cranberries demonstrated that variation in cranberry yield was primarily explained by variation in fruit set; berry enlargement was secondary and seed set was a minor factor (Baumann and Eaton, 1986). Hence, a grower's objective for pollinator service can be met when most cranberry flowers receive the minimum number of tetrads necessary to elicit fruit set. For the young plants used here, more than four and as many as eight tetrads were needed to yield a mature cranberry on the first pollinated flower of an upright shoot (Fig. 1). Comparisons of pollen receipt by stigmas revealed up to a sixfold difference between the mean loads deposited by different pollinator species during single floral visits (Fig. 4a). However, since 50–80% of individual bees deposited at least six tetrads during nonrobbing (legitimate) floral visits (Fig. 5), bee species differed far less in predicted fruit set than one would expect from the mean stigmatic pollen load. If all 5–6 flowers of a flowering shoot receive at least one legitimate visit from any of these bee species, then a mean of 3–5 flowers per shoot would receive enough pollen to elicit fruit set, a yield in excess of the commercial expectation of two fruits per flowering shoot (e.g., Kevan et al., 1983). The focus is thus shifted from relative pollinator efficacies to their visitation intensities, including forager fidelities to cranberry, their abundances on the bogs and likelihoods of stigmatic contact, and to some degree, bees' foraging tempos.

In contrast, evolutionary biologists interested in paternal fitness mediated by pollen tube competition should be more interested in bee species delivering excessive pollen loads. For cranberry plants growing under our conditions, stigmatic loads in excess of 16 tetrads did not enhance fruit or seed set. Therefore, not all pollen grains within generous stigmatic loads ($>$16 tetrads) could have sired seed; pollen generating the slowest growing tubes were presumably outcompeted in the race for ovule access. Only 17 of 100 single floral visits by honey bees delivered $>$16 tetrads, whereas 73% of 22 bumble bee visits delivered $>$16 pollen tetrads on the first visit. Of the four bee species studied here, bumble bees would seem to be the most important agents of inter-male competition in cranberries.

In this context, it is also worth noting that all of the manual pollinations in our study were cross-pollinations. Cranberry is self-fertile, but has been shown to mature somewhat more seed if cross-pollinated than if selfed, apparently owing to post-zygotic abortion (Sarracino and Vorsa, 1991). In that study, pollen was heaped manually onto stigmas, ensuring pollen tube competition. For the more modest pollen loads that most bees were found to actually deliver in this study, the benefits of outcrossing may be less evident in terms of seed set but more evident in terms of selective fruit abortion when maternal resources are limiting. This is a moot point for commercial cranberry bogs, which are propagated vegetatively from single cultivars, but may be relevant for clonal mosaics in the wild.

Fruit set and seed content of cranberries is strongly affected by flowering sequence within an upright flowering shoot; later flowers are less likely to set fruits, and late berries will be less seedy than those developing from earlier flowers (Baumann and Eaton, 1986). Precedence in maternal resource competi-

tion among developing fruits seems to be a common phenomenon among angiosperms (e.g., Galen et al., 1985; Helenurm and Schaal, 1996; Marshall et al., 1996). Earliest fruits often co-opt maternal resources, leading later fruits to abort (Stephenson, 1981). Four pollination factors may explain the phenomenon, three intrinsic to the plant (Figs. 1, 2, and 3). All relate to our pollination curves and should be discernible by our methods. The probability of fruit set may decline because (1) more pollen grains are needed to elicit any fruit set (the greater threshold shifts the entire curve to right); (2) the ratio between pollen deposited and seeds set increases (slope of curve flattens); or (3) more pollen is needed to maximize fruit set, because the asymptote has increased. These are all post-pollination phenomena, but they all impinge on measured pollination efficacies of pollinators, because larger stigmatic loads become more valuable for sexual reproduction. Temporal changes in pollinator service may also exist, such as declining visitation intensity or fewer visits delivering adequate pollen loads later in the season (Jennersten et al., 1988; Aizen, 2001). Comparisons of pollinator efficacies that use actual fruit or seed set can be confounded by differential abortion of later developing fruits owing to maternal resource limitation (e.g., Handel and Mishkin, 1984; Birrenkott and Stang, 1989). In such cases, variability in pollinator service can be better evaluated by comparing stigmatic pollen loads that have been translated into expected fruiting responses using dose–response curves such as the ones we have developed here for cranberry.

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